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Strategies for on-chip DNA processing on magnetic microbeads

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We present the detection of DNA concatemer products of rolling circle amplification (RCA) via optomagnetic (OM) measurements on functionalized magnetic nanoparticles (MNPs). Streptavidin coated magnetic microbeads (MMBs) were employed as a movable substrate to transport the biotinylated target and products between three microfluidic chambers corresponding to the reactions (Fig. 1A): (i) circularization of padlock probes (PLPs) to the target and enzymatic ligation of PLPs to form a circular template (58°C), (ii) RCA of circular templates to form a long single-stranded DNA concatemer[1] (37°C), and (iii) OM detection of depletion of MNPs upon binding to RCA products (anchored to MMBs) (52°C). For the entire assay, it is crucial that all reacted DNA probes are anchored to MMBs in the first step as otherwise fewer RCA products reach the detection chamber. We therefore tested and compared the following strategies for on-chip DNA ligation (Fig. 1A):

(1a) on-chip simultaneous circularization and ligation of PLPs to target and target capture on MMBs.

(1b) on-chip circularization and ligation of PLPs on targets and subsequent target capture on MMBs.

(1c) off-chip PLP circularization on targets followed by on-chip ligation and subsequent capture on MMBs.

(2) as in (1c) but targets are captured on MMBs functionalized with a DNA capture probe.

Fig. 1B shows the measured depletion of MNPs vs. time after the above procedure followed by RCA and OM quantification of the relative amount of free MNPS. In the simplest scenario (**1a**), there was no control over the sequence PLP hybridization → PLP ligation → capture on MMB, which led to an overall poor assay performance with capture of ≈10% of the free MNPs. For the sequential operations in cases **(1b)** and **(1c)**, a capture of ≈34% of the free MNPs with no significant difference between on-chip and off-chip PLP circularization. Upon introduction of the DNA capture probe on the MMBs rather than binding the target directly to the MMBs using the biotin-streptavidin bond in case **(2)**, we found that ≈80% of the free MNPs were captured on the RCA products. This improved performance is attributed to the release of the RCA products from the MMBs in the detection chamber, which reduces diffusion limitations.

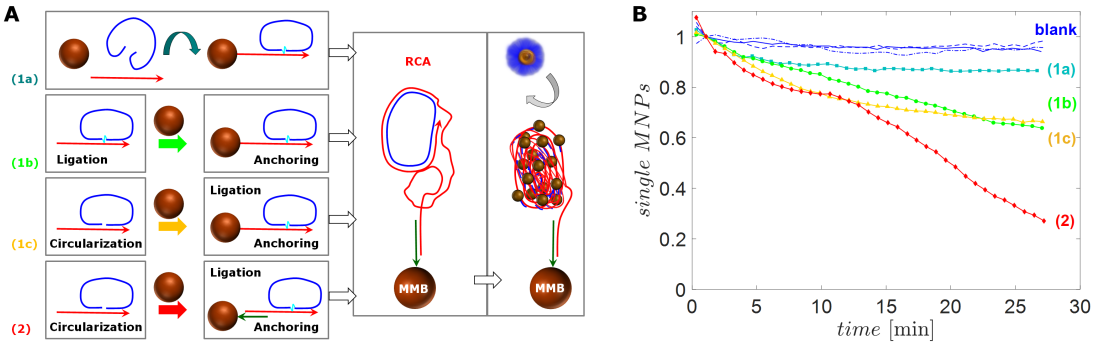

Figure 1: (A) Multi-step DNA processing using MMBs as movable substrate for capture, ligation, RCA and optomagnetic detection. The figure shows schematics of DNA ligation, RCA, and detection, where functionalized MNPs bind to multiple sites of the RCA product and (1a), (1b), (1c), and (2) illustrate the strategies for one-pot vs. sequential PLP circularization, ligation and capture on MMBs. (B) Time-traces of depletion of single MNPs in response to 200 pM target concentration and no-template controls.

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